NEW APPROACH METHODS AND REDUCING THE USE OF LABORATORY ANIMALS FOR CHRONIC AND CARCINOGENICITY TESTING

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Office of Chemical Safety and Pollution Prevention in collaboration with the Office of Research and Development

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New Approach Methods and Reducing the Use of Laboratory Animals for Chronic and Carcinogenicity Testing

1.0 Introduction

The mission of the Environmental Protection Agency's (EPA) Office of Chemical Safety and Pollution Prevention (OCSPP) is to protect humans and the environment from potential risks associated from exposure to pesticides and toxic chemicals. In order to achieve this, two offices within OCSPP are responsible for evaluating these potential risks. The Office of Pesticide Programs (OPP) regulates the use of all pesticide chemicals, while the Office of Pollution Prevention and Toxics (OPPT) evaluates new and existing chemical substances (excluding, among others, pesticides, tobacco and tobacco products, food, food additives, drugs, and cosmetics).

The EPA-OPP and EPA-OPPT regulate and evaluate chemicals under authority granted by regulatory statutes, which also allow the Agency to require or request a suite of data and relevant information from pesticide registrants and chemical manufacturers to support scientifically-based risk assessment. To assess the potential hazard of a chemical for human health risk assessment, toxicological studies in laboratory animals are used to provide information on a wide range of adverse health outcomes, routes of exposure, exposure durations, and lifestages. Both EPA-OPP and EPA-OPPT routinely request chronic and/or carcinogenicity testing to be conducted in laboratory animals. These laboratory animal studies have been used by regulatory agencies for decades to evaluate hazards and dose-response and for extrapolating risk through the development of points of departure and reference doses. However, the rodent bioassay has some well-recognized shortcomings, including imperfect translational relevance to human health, extensive time required to conduct studies and report results, high cost, low throughput, and the large number of animals required (typically > 1000 per substance).

New approach methodologies (NAMs) is a broadly descriptive reference to any technology, methodology, approach (including computational/*in silico* models (i.e., QSARs)), or combination thereof that can be used to provide information on chemical hazard and risk assessment that avoids the use of intact animals. In September, 2019, EPA Administrator Andrew Wheeler directed staff and managers at the agency to prioritize the reduction of animal use and specifically noted that EPA will reduce its requests for, and our funding of, mammal studies by 30 percent by 2025 and will eliminate all mammal study requests and funding by 2035 (unless approved by the Administrator on a case-by-case basis). To achieve these goals, EPA must accelerate NAM and other related animal reduction activities.

EPA's OCSPP is committed to supporting NAM development and implementation and, to this end, is collaborating with the Office of Research and Development (ORD) and numerous government, industry and non-governmental stakeholders on a variety of projects such as retrospective analyses and NAM development for a range of human health and ecological effects. As summarized in this draft white paper and described in detail in attachments and appendices, OCSPP is working to improve chronic/carcinogenicity testing through collaborative projects with the Division of the

National Toxicology Program (DNTP), the Health and Environmental Sciences Institute (HESI), the PETA International Science Consortium Ltd. (PETA-ISC), and pesticide and chemical companies. All of these activities are currently on-going and are at a point where input from external experts and the public would be helpful. OCSPP will solicit comment from the Science Advisory Board (SAB) through a consultation on these activities.

1.1. Regulatory Background

Under 40 CFR Part 158, the EPA-OPP requires toxicology data to support registration of food and non-food use pesticides. The regulations give EPA substantial discretion to make registration decisions based on what the Agency believes are the most relevant and important data for each action. The actual data and studies required may be modified on an individual basis to fully characterize the use and properties of specific pesticide products under review. Also, the data requirements may not always be considered appropriate. For instance, the properties of a chemical or an atypical use pattern could make it impossible to generate the required data or the data would not be considered useful in the Agency's evaluation. Therefore, the Agency may waive data requirements, but must ensure that sufficient data are available to make the determinations required by the applicable statutory standards. The 40 CFR also provides EPA with broad flexibility under 158.75 to request additional data beyond the Part 158 data requirements that may be important to the risk management decision. Alternative methods and approaches can be considered and accepted for these additional data, when appropriate.

EPA-OPP has developed a 2009 Strategic Vision for Adopting 21st Century Science Methodologies[1, 2]. This strategic direction describes the need for a broader suite of computer-aided methods to better predict potential hazards and exposures, and to focus testing on likely risks of concern; improved approaches to more traditional toxicity tests to minimize the number of animals used while expanding the amount of information obtained; and improved understanding of toxicity pathways to allow development of non-animal tests that better predict how exposures relate to adverse effects. EPA-OPP's commitment to reducing the use of animals in pesticide testing and accelerating the use of alternative methods was reaffirmed in 2016 in an open letter to stakeholders from EPA-OPP's Office Director that describes the need to re-evaluate studies not routinely used in decision making, advancing the implementation of NAMs and to facilitate global harmonization of new approaches[3].

Under Section 4 of the Toxic Substances Control Act (TSCA), the EPA-OPPT is authorized to require testing to develop data about health or environmental effects when there is insufficient data to determine whether a chemical presents an unreasonable risk to health or the environment. A new subsection under Section 4 also requires the EPA to develop a strategic plan to promote the use and development of alternative test methods and strategies to reduce, refine or replace vertebrate animal testing (Section 4(h), Reduction of Testing on Vertebrates). Under this new subsection, when testing is required, EPA "shall reduce and replace, to the extent practicable, scientifically

[1] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/strategic-vision-adopting-21st-century-science
[2] [HYPERLINK "https://www.regulations.gov/document?D=EPA-HQ-OPP-2011-0284-0006"]
[3] [HYPERLINK "https://www.regulations.gov/"]

justified, and consistent with the policies of this title, the use of vertebrate animals in the testing of chemical substances or mixtures...." (Section 4(h)(1)).

In 2018, EPA-OPPT published its Strategic Plan to Promote the Development and Implementation of Alternative Test Methods within the TSCA Program[4]. The TSCA Strategic Plan has three core components: (1) identifying, developing and integrating NAMs for TSCA decisions; (2) building confidence that the NAMs are scientifically reliable and relevant for TSCA decisions; and (3) implementing the reliable and relevant NAMs for TSCA decisions.

1.2 Scope & Purpose of the 2020 Science Advisory Board Meeting on NAMs for Chronic/Carcinogenicity Testing

This draft white paper provides a brief overview of activities focused on different aspects of improving the science used in risk assessment for chronic/carcinogenicity testing. These activities are organized by the 3Rs principles for laboratory animal testing-- *reduce, replace, refine* as originally proposed by Russell and Burch (1). Because of the complexities in biology and toxicology, there will not be a "one-size-fits-all" solution to improving chronic/carcinogenicity testing. As such, EPA and its collaborators are taking a multifaceted approach that advances several areas simultaneously.

Reduce: using fewer animals in experimentation.

OPP has been collaborating with PETA-ISC along with Canada Pest Management Regulatory Agency (PMRA), Australian Pesticides and Veterinary Medicines Authority (APVMA), and several pesticide registrants to develop a risk-based weight of evidence (WOE) analysis framework specifically for use in evaluating the need for chronic/carcinogenicity testing in rodents for pesticide active ingredients. This project is called Rethinking Carcinogenicity Assessment for Agrochemicals Project (ReCAAP) and is described in Section 2.0 below. The draft WOE approach is conceptually consistent with existing EPA-OPP waiver guidance for neurotoxicity, inhalation, dermal and immunotoxicity studies[8]. Attachment 1 provides the current working draft of a risk-based WOE waiver framework. Some initial case studies have been done as part of WOE waiver framework development phase of the project; one example draft case study is provided in Attachment 2. Another set of case studies are just getting started and will be used to test the draft WOE waiver framework. Feedback from the SAB and public will be useful for revising the WOE waiver framework and case studies.

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Replace: using non-vertebrate animal test systems

To achieve Administrator Wheeler's goals to eliminate all mammal study requests and funding by 2035, more NAM approaches need to be developed to replace the typical laboratory animal studies. Beyond the ethical issues associated with ~~reducing~~ animal use, NAMs are expected to improve the

Commented [GH2]: Wording is confusing: "ethical issues in reducing animal use"? I read this as ethical issues in animal testing (not reducing testing)...

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[4] [HYPERLINK "https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/strategic-plan-reduce-use-vertebrate-animals-chemical"]

scientific foundation of risk assessments by providing more human-relevant information that is more efficient and less costly. To this end, EPA is collaborating with Division of the National Toxicology Program (DNTP) of National Institute of Environmental Health Sciences (NIEHS) and HESI to consider NAM-based approaches to replace chronic/carcinogenicity testing in mammals. These efforts are in the early stages of development and would benefit from expert and public input on the direction and scope of these efforts.

- The DNTP is an intramural research division within the NIEHS. The DNTP is beginning a "Translational Toxicological Pipeline" approach that includes data mining, computational toxicology, *in vitro* and *in vivo* testing. This involves an initiative called Health Effects Innovations (HEIs), which will concentrate research and translation efforts in three areas, one of which is carcinogenicity testing for the 21st century (the other two focusing on cardiovascular disease and developmental neurotoxicity). The carcinogenicity HEI is described below in Section 3.1.

- The nonprofit, Health and Environmental Sciences Institute's Emerging Systems in Toxicology (HESI eSTAR) program has convened a multi-sector and multi-disciplinary working group to build and implement a framework for the application of a transcriptomic evaluation of chemical exposure for use in risk assessment to result in a health protective point of departure (POD). Section 3.2 describes the foundation and goals for this work.

Commented [CW([3]: We have presentations from our Board of Scientific Counselors meetings but no publications.

Commented [LA4]: Do we have attachments for these? None is fine, just need to number the KMD ones.

Refine: the lessening or avoidance of pain and distress.

Despite efforts to develop alternatives to animal testing and providing waivers for those studies determined not to be needed, some laboratory animal studies will continue to be conducted in the near future. EPA is also engaged with stakeholders in activities to adopt kinetically-derived maximum dose (KMD) approach in lieu of the traditional maximal tolerated dose (MTD) in designing mammalian toxicity studies. When using the KMD, toxicity studies are conducted at kinetically linear doses or slightly above the point of inflection from linearity of kinetics. The KMD approach can help prevent the use of toxicity data generated at excessively high doses at which the physiology of test species is greatly altered, and the levels are orders of magnitude higher than possible human exposures. Thus, the KMD approach not only lessens or avoids unnecessary pain and distress in animals, but also generates data that are relevant and more predictive of human health risks. EPA-OPP has already received multiple submissions of KMD proposals from pesticide registrants and interest in this approach is growing. There is an immediate need to standardize these approaches for broad regulatory use and facilitate global harmonization. EPA-OPP has been collaborating with Canada PMRA, APVMA, HESI, the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and several pesticide registrants on KMD. Section 4 below describes these activities in more detail. Attachment X provides an example of a KMD approach accepted by EPA-OPP in 2019. Attachments Y and Z describe a new workgroup being formed by HESI and workshop being planned for October, 2020 both aimed at the development of best practices for KMD.

2.0 Reduce: Develop Weight of Evidence Approach for Waiving Chronic/Carcinogenicity studies

As described in the 40 CFR Part 158, EPA-OPP requires extensive toxicology testing for pesticides in a variety of species (e.g., rat, mouse, rabbit, dog, fish, bird, insects) to conduct human health and ecologic risk assessments. Carcinogenicity studies in two rodent species are required for the registration of food use pesticides or when the use of a pesticide is likely to result in repeated human exposure over a considerable portion of the human lifespan (40 CFR Part 158.500). Agency guideline carcinogenicity studies (OCSPP 870.4200, 870.4300) have been harmonized with OECD guidelines (Test Nos. 451 and 453) and are designed to test three or more doses in both sexes (with at least 50 animals/sex/dose) with adequate dose spacing to characterize tumor dose-response relationships. Test substances are typically administered in animal carcinogenicity studies by the oral route for food use pesticides but can also be conducted via dermal or inhalation route on a case-by-case basis. These studies are generally conducted in mice and rats with exposure durations of 18-24 months for mice and 24 months for rats, which represent exposures of the majority of the expected lifespan in these animals.

However, EPA-OPP has flexibility in implemented these data requirements. EPA-OPP's 2013 "Guiding Principles for Data Requirements" guidance[5] was developed to provide consistency in the identification of data needs, promote and optimize full use of existing knowledge, and focus on the critical data needed for risk assessment while avoiding unnecessary use of time and resources, data generation costs, and animal testing. The guiding principles document ensures that "there is sufficient information to reliably support registration decisions that are protective of public health and the environment while avoiding the generation and evaluation of data that does not materially influence the scientific certainty of a regulatory decision...."

In an effort to reduce the number of animals used for pesticide toxicity testing, EPA-OPP created the Hazard and Science Policy Council (HASPOC) to review requests for waiving animal study requirements for human health risk assessments and make recommendations based on a weight-of-the-evidence approach. To date, HASPOC has primarily focused on study waivers for neurotoxicity studies, subchronic inhalation and dermal toxicity studies, and immunotoxicity studies but has also waived some developmental neurotoxicity, developmental toxicity, reproductive toxicity and chronic studies. The waiving of studies by HASPOC has resulted in over 200,000 animals saved (2). This has been accomplished through the implementation of the guidance document entitled "Part 158 Toxicology Data Requirements: Guidance for Neurotoxicity Battery, Subchronic Inhalation, Subchronic Dermal and Immunotoxicity Studies[6]." This guidance document describes a risk-based WOE approach that considers physical-chemical properties, exposure profile, toxicity profile, mode of action/adverse outcome pathway, read across along with other relevant information. EPA-OPP is now extending this effort to identify the types of data that can be used in a WOE framework to inform chronic/carcinogenicity testing without conducting one or more of the rodent cancer bioassays.

The ReCAAP was established to help develop a draft WOE waiver framework and is led by PETA International Consortium Ltd., and includes experts from EPA-OPP and other US agencies, Canada PMRA, APVMA and industry. The problem statement and objectives of ReCAAP are excerpted below.

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[5] http://www.epa.gov/pesticides/regulating/data-require-guide-principle.pdf

[6] [HYPERLINK "http://www.epa.gov/pesticides/regulating/part158-tox-data-requirement.pdf"]

> *"There are no specific criteria to determine when not to require the Combined Chronic Toxicity/Carcinogenicity studies (OECD 453; 451), or how to determine appropriate POD for chronic risk assessments for pesticides based on available toxicological and exposure data in the absence of chronic toxicity studies ~~studies~~..... there is a movement to transition away from a routine 'check-box' approach towards a more scientifically sound weight of evidence (WOE) carcinogenicity assessment for non-genotoxic food-use pesticides."*

At this time, ReCAAP has developed a draft risk-based WOE ~~framework~~approach ~~which~~ which is provided in Attachment 1. The major components are:

- Use pattern & exposure scenarios
- Physical-chemical properties
- Pharmacokinetics (PK) and absorption, distribution, metabolism and excretion (ADME)
- Toxicity profile (mode of action; acute and subchronic toxicity; potential for hormone perturbation or immune suppression; genetic toxicology; mechanistic evidence from key event studies, biomarkers, NAM information, etc.)
- Read across
- Proposed Points of Departure and Prospective Risk Assessments
- Summary & recommendations for/against waiver

ReCAAP has also developed some initial case studies that were developed as part of framework development stage. One example is provided in Attachment 2. Another set of case studies are just being started and will be used to test the draft WOE waiver framework. Feedback from the SAB and public will be useful as the project continues to move forward.

3.0 Replace: Developing NAMs for Chronic/Carcinogenicity Testing

The effort to develop NAMs for chronic/carcinogenicity testing is one of many areas that EPA-OPP and EPA-OPPT are working with stakeholders ~~on~~. EPA-OPPT has a long history of using QSARs and read across approaches in its new chemicals program for hazard identification and fate characterization and modeling for exposure assessment. As part of the TSCA Strategic Plan, a list of NAMs accepted by EPA-OPPT for use in decision making is available[7]. EPA-OPP has been actively engaged in the development and implementation of NAMs for acute lethality, skin irritation, and eye irritation for the last several years. In addition, EPA-OPP and EPA-OPPT jointly developed the "Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing[8]." EPA-OPP and EPA-OPPT are also working together to implement NAMs for inhalation toxicity and solicited comment from the FIFRA Scientific Advisory Panel in 2018 on a case study using the fungicide, chlorothalonil[9].

[7] [HYPERLINK "https://www.epa.gov/sites/production/files/2019-12/documents/alternative_testing_nams_list_first_update_final.pdf"]
[8] [HYPERLINK "https://www.regulations.gov/document?D=EPA-HQ-OPP-2016-0093-0090"]
[9] [HYPERLINK "https://www.epa.gov/pesticides/december-4-6-8-2018-fifra-sap-meeting"]

3.1 DNTP Efforts to Improve Carcinogenic Assessment of Environmental Substances

The Division of the National Toxicology Program (DNTP) is an intramural research division within the National Institute of Environmental Health Sciences (NIEHS). The DNTP is committed to advancing public health and the discipline of toxicology through the use of innovative tools and strategies that are translatable, predictive, and timely. This commitment requires the continual evaluation and evolution of existing methodologies as well as the development and application of new technologies and approaches with the potential to protect and improve public health. This new "Translational Toxicological Pipeline" approach spans the continuum of data mining, computational toxicology, bioactivity screening, *in vitro* studies, short-term *in vivo* studies, chronic *in vivo* studies, and knowledge integration. Earlier access to information and less reliance on chronic *in vivo* studies is anticipated given the tiered structure of this approach. The major vehicle for this paradigm shift at DNTP is a new initiative called HEIs, which will concentrate research and translation efforts in three areas, one of which is carcinogenicity testing for the 21st century (the other two focusing on cardiovascular disease and developmental neurotoxicity).

Since the early 1980s, the US National Toxicology Program has served as a national and world resource for conducting the two-year rodent bioassay to assess the carcinogenic potential of industrial and agricultural chemicals, food additives, environmental pollutants, and other exposures of public health concern. The current National Toxicology Program rodent cancer bioassay evolved from that of the National Cancer Institute in the 1970s. Since its inception, DNTP has published technical reports on over 600 substances, providing valuable cancer hazard information for the protection of public health. In addition, these studies represent a unique and valuable source of reference data to support the development new assays that can potentially better inform cancer risk assessment. The Carcinogenicity HEI (Carci HEI) will work with federal agencies and other stakeholders to develop efficient, fit for purpose approaches to characterize the potential for environmental exposures to cause or contribute to the development of cancer in humans and communicate actionable information to stakeholders in a timely manner. The effort is divided into three focus areas, as described below.

1. Developing a translational toxicology pipeline (TTP) of capacities to characterize the potential for environmental substances to cause or contribute to the development of cancer. This approach will evaluate opportunities to assess carcinogenic potential using innovative methods and approaches while maintaining the 2-year rodent bioassay as an option in the TTP. The TTP will initially be populated using existing tools, technologies, and animal studies for which existing data are available for chemicals previously evaluated in the NTP rodent bioassay. An Adverse Outcome Pathway (AOP) framework will be employed in combination with Integrated Approaches to Testing and Assessment (IATAs), considering data from various components of the TPP in combination. IATAs will be iteratively developed and evaluated against results obtained from existing data from NTP bioassays, with each cycle informing potential modifications to improve their predictive performance.

Commented [GH5]: Should this be consistent with translational toxicology pipeline (TTP)? If so, all need corrections that follow.

2. Establishing fit-for-purpose TPPs to characterize the potential for environmental exposures to cause or contribute to cancer development of site-specific cancer in humans. Recognizing that "cancer" is a collection of over 100 related diseases that may evolve by different pathways, a disease-specific TPP framework will be developed using

approaches that address mechanisms relevant to site-specific cancers and their link to environmental exposures. Initial focus would be on those cancers with a high or increasing burden of disease in the human population, such as early-onset colorectal cancer.

3. Developing a communication strategy that will engage stakeholders and maximize the impact of the Carci HEI. The identification of environmental causes of cancer is of great potential benefit to public health. However, such information is only of value if communicated in a manner that is relatable to the target stakeholder group, therefore requiring a unique communication effort for different audiences. The Carci HEI will work with communication experts and stakeholders to develop a strategy that will help ensure clear and actionable information is provided to all audiences in a timely manner.

The next steps planned by the Carci_HEI for 2020 include:

- engaging in further dialogue with stakeholders to better understand and publish their specific requirements, data needs, and contexts of use;
- providing easy access to historical NTP bioassay data in a computational-ready format;
- identify initial human cancer areas of focus and availability of relevant assays/models;
- evaluating the concordance between human and rodent cancer mutational signatures;
- evaluating the concordance between *in vivo* and *in vitro* (human and rodent) mutational signatures associated with environmental exposures;
- evaluating the association between the Key Characteristics of Cancer and outcomes in NTP rodent bioassays for chemicals in the Tox21 inventory.

Progress on each of these initiatives will be publicly communicated and made available on the NTP website.

3.2 Health and Environmental Sciences Institute (HESI) Point of Departure Program Overview

A cornerstone of traditional human health risk assessment is the evaluation of dose-response and temporality of toxic effects in experimental animal studies, resulting in the identification of points of departure (POD) and subsequent derivation of non-cancer or cancer values. A major limitation in this traditional approach to human health risk assessment is the necessity for resource-intensive studies, using whole laboratory animal models, to identify PODs based upon apical adverse effects (e.g., histopathological manifestations; organ/tissue weights; etc.) (3.4). The justification for continued use of this low-throughput approach is born of historical context that the basis of a risk assessment be anchored to identification of a POD from *in vivo* apical effects. Development and application of alternative approaches that facilitate higher-throughput POD identification is important to advancing assessment of chemicals and pesticides.

Several alternative approaches to identify PODs based on non-apical endpoint bioactivity are under development. These approaches cover a diverse array of model systems (both *in vitro* and *in vivo*) and incorporate the use of *in silico* and molecular technologies (e.g., high throughput screening, structure activity, and an assortment of omic technologies) (5-11). Several independent investigators have explored the use of short-term (e.g., 5 days), repeat dose studies in rodents to derive molecular, or more specifically, transcriptomic (i.e., genomic or subgenomic level changes in mRNA levels) PODs (12-17). These studies have found that transcriptomic-based PODs closely align with PODs derived from traditional apical endpoints observed in guideline toxicological studies. Not only do short-term transcriptomic PODs align with traditional endpoint (e.g., histopathology)-based PODs in the same short-term exposure paradigm, these also closely align with PODs that are identified in subchronic or chronic duration guideline toxicological studies such as the 2-year rodent cancer bioassay. Notably, across two different rodent species, sexes, and routes of exposure, these 'omic studies, although using a variety of transcriptome measurement technologies, have arrived at the same conclusion of apical endpoint and molecular bioactivity POD concordance. These findings highlight the generalizability of the approach and the reliability/accuracy of the measurement technologies and analysis methods (18-22). Such observations are consistent with the logic flow illustrated in the Adverse Outcome Pathway concept that a molecular level change precedes a change at the organ/tissue level, such as histopathology. Therefore, molecular level changes (using a comprehensive analysis method such as transcriptomics) are a plausible means for identifying human health-protective bioactivity-based POD values.

One potential challenge associated with a transcriptomic based approach to deriving a POD is that it departs from the traditional toxicity testing and assessment paradigm, as it does not entail the direct identification of specific adverse effects. Instead, the short-term (e.g., 5-day) *in vivo* exposure transcriptomic approach leads to identification of a dose (e.g., POD) associated with minimal gene expression change. Due to our limited understanding of systems biology, often the genes underlying the transcriptomic POD do not have an immediately discernable mechanistic linkage to a specific toxicological pathway or process. Although the landscape of molecular perturbations induced by environmental chemicals are complex, the noted observations of concordance between transcriptomic and apical PODs opens the door to the idea that it is unnecessary to have a known mechanistic linkage between transcriptional changes and specific adverse effects to identify a POD that is sufficient for human health risk assessment. In practice, the comprehensive nature of a transcriptomic-based POD may be even more protective of human health than the current toxicity testing and risk assessment process which queries numerous but not all potential hazards. Importantly when this challenge of translation to apical toxicological effects is viewed through the lens of systems biology it is plausible to hypothesize that the genes that underlie an apical effect POD are closely coregulated (i.e., similar potency) with genes informative of molecular level toxicological processes (23). Bearing this in mind there is a definable route to linking transcriptomic changes to toxicological apical effect processes and to support mechanistic inquiry; however, from purely an empirical perspective, it is unnecessary to delineate these mechanistic processes in order to identify a bioactivity-based transcriptional POD that can be used in risk assessment.

Despite the empirical support for the use of an *in vivo* transcriptional POD in risk assessment and the obvious efficiency in the approach compared to traditional guideline study-based animal testing, there are still some important questions/uncertainties that need to be addressed before a transcriptional POD could be considered for regulatory use of chemical toxicity assessment. Some

of these issues relate to study design, data analysis, POD modeling/identification, appropriate uncertainty factor application for non-cancer value derivation, and context of use of such values. The objective of this HESI project is to identify and address the scientific uncertainties associated with the use of a transcriptomic POD in human health risk assessment and to develop a framework for application of the approach in human health risk assessment.

The nonprofit, Health and Environmental Sciences Institute's Emerging Systems in Toxicology (HESI eSTAR) program has convened a multi-sector and multi-disciplinary working group to build and implement a strategy for progressing the science in this area. The working group is currently comprised of approximately 30 scientists and stakeholders from the private, public and academic sectors, representing multiple countries. This includes several scientists from the US EPA. To date the working group has met several times and performed a problem formulation exercise that resulted in the following problem statement and goal:

> *"In order to reduce the need for resource intensive animal testing we propose the development of a framework for the application of a transcriptomic evaluation of chemical exposure for use in risk assessment to result in a health protective POD with confidence, without needing to correlate with a specific adverse effect, mechanism and/or mode of action."*

Based upon identification of this specific challenge, the HESI working group has proposed the following goal:

> *"To address uncertainties about the derivation and use of transcriptomics-based molecular PODs from a relevant in vivo study; and, to demonstrate application of these transcriptomic PODs to human health assessment of chemicals."*

To date there are two proposed work products that the working group will produce. The first will be a manuscript that reviews the state of the science and defines remaining uncertainties that may hinder the adoption of an *in vivo*,

Commented [LA6]: what about the second one?

4.0 Refine: Kinetically-Derived Maximum Dose (KMD)

PK refers to the study of chemical movement into, through, and out of a living organism determined by the rate and extent of a chemical's ADME. ADME determines the amount of chemical available for eliciting toxicological response in the tissues from a given external exposure. Thus, knowledge of PK, alone with other information (e.g., mode of action), can improve the design of animal toxicity studies and interpretation of results from these studies. EPA has used PK data and information to replace default uncertainty factors (e.g., using comparative metabolism data, *in vitro* absorption rate measurement, PBPK model predictions) for more refined, data-driven risk assessment. Another use of PK data and information is adopting the KMD approach in lieu of the traditional MTD approach when selecting top doses in animal toxicity studies. KMD refers to the highest dose at, or slightly above the point of departure from dose proportionality, or PK linearity. This approach is endorsed in the OECD GD116:

> "Although top dose selection based on identification of inflection points in toxicokinetic nonlinearity may result in study designs that fail to identify traditional target organ or body weight effects, it must be appreciated that metabolic saturation in fact represents an equivalent indicator of biological stress. In this case, the stress is evidenced by appearance

of non-linear toxicokinetics rather than appearance of histological damage, adverse changes in clinical chemistry, hematology parameters or decrease in body weight gain."

Toxicity findings at doses above a KMD (i.e., where PK is non-linear) may not be relevant to human health risks when potential exposure levels are orders of magnitude lower. Such high dose levels may greatly alter the physiology of animals and overwhelm adaptive or repair mechanism. Non-linear PK can be caused by saturation or limitation of various ADME factors, such as absorption in gut wall, plasma protein binding, metabolic capability, and active renal secretion.

While KMD is routinely considered in preclinical tests to provide perspective on the relevance of study results to human safety assessment for drugs, KMD is rarely used in chemical safety assessment. In the last few years, EPA-OPP has received multiple submitted studies using or proposing a KMD approach and the interest in this approach is growing rapidly. The quality of the KMD submissions has varied significantly. As such, there is need to develop more consistent, transparent approach that supports broader, accelerated application and global harmonization.

EPA is engaging international stakeholders to develop best practices on KMD analysis. EPA, NICEATM, and HESI are co-organizing a 3-day workshop, on October 6-8, 2020 to address commonly raised technical and science issues related to the use of KMD as an approach to set top dose in toxicity studies, or to interpret dose-response results from toxicity studies. In addition, a working group has been formed to develop best practices (1) selecting the appropriate PK parameter to examine dose proportionality; (2) estimating the onset of non-linear PK based on PK data or predictions; (3) conducting statistical analyses to determine a KMD for interpreting dose-response data; and (4) determining and using a KMD to set the top dose in toxicity studies.

The following attachments are provided the SAB for consideration:

- Two case studies
- Agenda and description of the upcoming workshop entitled XXXX
- Workgroup charge for the HESI workgroup on XXXX

Commented [LA7]: T? What other one—maybe one that didn't work? We'll need to hide the chemical names.

5.0 Summary & Next Steps

EPA is actively engaged with numerous stakeholders to reduce laboratory animal used in chronic/carcinogenicity testing and develop NAMs for use in risk assessment. ~~Some of these activities involve chronic/carcinogenicity testing.~~ EPA will solicit comments from the SAB on direction, scope and/or status of the on-going projects related to moving away from the traditional laboratory animal chronic/carcinogenicity bioassay. The feedback from the SAB will be used to inform the next steps on each activity as the agency moves towards adopting new scientific approaches.

6.0 References

[ADDIN EN.REFLIST]